MICROALGAE *SCENEDESMUS* SP. BIOREACTOR FOR REMOVAL OF NUTRIENTS AND ORGANIC CONTENTS FROM WET MARKET WASTEWATER

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DEDICATION

I dedicated this work to my beloved parents,
JAIS BIN MARJAN and ROSNAH BINTI MOHD LATIM
my best supervisor, Assoc. Prof. Dr. Radin Maya Saphira Radin Mohamed,
siblings and friends
for giving me infinite care and blessing
thank you for your endless support to me…

Love,
-Noor Maisara Jais-

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ABSTRACT

Wet market allows customers to get fresh foodstuff. However, its wet market wastewater (WMWW) which contains COD, BOD, TSS, NH_3-N and P is commonly discharged directly into drainage without any treatment. Hence, the main aim of this research is to develop a prototype of *Scenedesmus* sp. bioreactor that can carry out phycoremediation process and ensure the parameters in WMWW can reach allowable standard. 16 types of samples based on four different initial concentration of *Scenedesmus* sp. (1×10^6 cell/mL, 10×10^6 cell/mL, 100×10^6 cell/mL, 1000×10^6 cell/mL) with presence or absence of agitator were optimized. The best growth rate and the highest reduction of nutrients and organic contents was chosen as optimum initial concentration of *Scenedesmus* sp. to be used in bioreactor. *Scenedesmus* sp. bioreactor was developed properly with four specific tanks and auto sensors for time and water level. The size is 15 cm (length) × 15 cm (width) × 30 cm (height) for each tank. Results showed that 100×10^6 cell/mL *Scenedesmus* sp. with agitated condition during optimization gave highest efficiency removal COD, BOD, NH_3-N and P which are >91% with highest growth rate 0.667 μ/day and 2.71×10^9 cell/mL biomass production. The concentration of COD, BOD, NH_3-N and P using *Scenedesmus* sp. bioreactor with ($p<0.05$) are pass sewage effluent Standard B. Reaction rate constant, k = 29.50 mg NH_3-N /mg chl-a per day and k= 22.12 mg PO_4^{3-}/mg chl-a per day and saturation constant, K_m= 227.68 mg/L of NH_3-N and K_m= 279.00 mg/L of PO_4^{3-} with Y_N= 0.017 mg chl-a/mg NH_3-N and Y_p= 0.054 mg chl-a/mg PO_4^{3-}. Thus, these expressions suggested that PO_4^{3-} produced three time more chl-a than NH_3-N produced chl-a during phycoremediation process by *Scenedesmus* sp. The outcomes from this study suggested that the *Scenedesmus* sp. bioreactor system is effective with >96% for the organic contents and nutrients removal from WMWW. This study may reduce environmental problem especially for aquatic and beneficial to authorities to produce national guiding principle of WMWW treatment.

ABSTRAK

Pasar basah merupakan tempat pelanggan untuk mendapatkan makanan segar. Walau bagaimanapun, air sisa daripada pasar basah (WMWW) yang mengandungi COD, BOD, TSS, NH_3-N dan P biasanya dilepaskan terus ke dalam saliran tanpa sebarang rawatan. Oleh itu, matlamat utama kajian ini adalah untuk membina contoh dasar bioreaktor *Scenedesmus* sp. yang boleh menjalankan proses fikopemulihan dan memastikan parameter dalam WMWW boleh mencapai piawaian yang dibenarkan. 16 jenis sampel berdasarkan kepada empat jenis kepekatan awal *Scenedesmus* sp. (1×10^6 sel/mL, 10×10^6 sel/mL, 100×10^6 sel/mL, 1000×10^6 sel/mL) dengan kehadiran atau ketiadaan pengaduk telah dioptimumkan. Kadar pertumbuhan terbaik dan pengurangan nutrien dan kandungan organik yang tertinggi telah dipilih sebagai kepekatan awal optimum untuk mikroalga *Scenedesmus* sp. untuk digunakan di dalam bioreaktor. Bioreaktor *Scenedesmus* sp. yang telah dihasilkan terdiri daripada empat buah tangki dan sensor automatik untuk mengukur masa dan jumlah air. Saiz untuk setiap tangki adalah 15 cm (panjang) × 15 cm (lebar) × 30 cm (tinggi). Keputusan menunjukkan bahawa 100×10^6 sel/mL dengan kehadiran pengaduk semasa pengoptimuman memberikan kecekapan yang tertinggi kepada COD, BOD, NH_3-N dan P iaitu >91% dengan kadar pertumbuhan tertinggi sebanyak 0.667 μ/hari dan 2.71×10^9 sel/mL untuk pengeluaran biojisim. Kepekatan COD, BOD, NH_3-N dan P dengan ($p<0.05$) berada di bawah piawaian kumbahan yang dibenarkan oleh Standard B. Kadar tindak balas berterusan, k = 29.50 mg NH_3-N /mg chl-a sehari dan k = 22.12 mg PO_4^{3-} / mg chl-a sehari dan ketepuan malar, K_m = 227.68 mg/L NH_3-N dan K_m = 279.00 mg/L PO_4^{3-} dengan Y_N = 0.017 mg chl-a / mg NH_3-N dan Yp = 0.054 mg chl-a / mg PO_4^{3-}. Oleh itu, penyataan ini mencadangkan bahawa PO_4^{3-} menghasilkan tiga kali chl-a berbanding daripada NH_3-N menghasilkan chl-a semasa proses rawatan air menggunakan *Scenedesmus* sp. Keputusan daripada kajian ini mencadangkan bahawa sistem bioreaktor *Scenedesmus* sp. berkesan untuk penyingkiran kandungan organik dan nutrien daripada WMWW. Kajian ini boleh mengurangkan masalah pencemaran

terutama kepada akuatik dan berguna kepada pihak berkuasa untuk menghasilkan panduan kebangsaan terhadap rawatan air sisa pasar basah.

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LIST OF SYMBOLS AND ABBREVIATIONS

WMWW	Wet market wastewater
COD	Chemical oxygen demand
BOD	Biochemical oxygen demand
TSS	Total suspended solid
O & G	Oil and grease
TN	Total nitrogen
PO_4	Phosphate
TP	Total phosphorus
NH_3	Ammonia
PO_4^{3-}	Orthophosphate
TOC	Total organic carbon
DOE	Department of Environment
Cd	Cadmium
Cu	Copper
Zn	Zinc
Fe	Iron
Co	Cobalt
Mg	Magnesium
N_2	Nitrogen gas
NO_3^-	Nitrate
NH_4	Ammonium
P	Phosphorus
CO_2	Carbon dioxide
HCO_3^-	Bicarbonate
CO_3^{2-}	Carbonate
IC	inorganic carbon
PSN	National Science Centre
TPM	Technology Park Malaysia

ASEAN	Association of Southeast Asian Nations
R & D	Research and Development
KAP	Klamath Algae Product
CTAB	Cetyl trimethyl ammonium bromide
CIA	Chloroform isoamyl alcohol
PVP	Polyvinylpyrrolidone
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
BLAST	Basic local alignment search tool
BBM	Bold Basal Medium
dH_2O	Distilled water
SESD	Science and Ecosystem Support Division
SGR	Specific growth rate
NH_3-N	Ammonia nitrogen
k	Saturation constant
K_m	Coefficient of kinetics

CHAPTER 1

INTRODUCTION

1.1 Research background

Many markets are now available for buyers to get fresh foodstuff such as wet markets and supermarkets. Although the wet market is traditional, it is still popular because of the price and quality of foodstuff is better than the wet market in supermarkets (Szeki, 2008). This WMWW has high content of chemical oxygen demands (COD) 375–1440 mg/L, biochemical oxygen demands (BOD) 68–295 mg/L, total suspended solid (TSS) 60–900 mg/L, oil and grease (O & G) 3.67–72 mg/L, total nitrogen (TN) 12.4–72.9 mg/L and phosphate (PO_4) 0.7–44.5 mg/L (Ghani, 2011; Zulkifli & Khalkausar, 2011). Unfortunately, the wet market wastewater (WMWW) is discharged directly into the drainage without any treatment cause harmful to aquatic live and environment.

In worldwide, there are wastewater treatment by using microalgae, known as phycoremediation technology. This phycoremediation is one of the commercially used treatments because it is cheap and easy to handle since years ago (Abdel-Raouf, Al-Homaidan & Ibrahim, 2012). This type of treatment was attractive due to the capabilities of microalgae during photosynthesis process and their ability to incorporate with nutrients such as nitrogen and phosphorus which may cause eutrophication if the wastewater contains excessive amount of this nutrients (Abdel-Raouf *et al.,* 2012; Jalal *et al*., 2011). Abdel-Raouf *et al.,* (2012) also stated that some microalgae such as *Scenedesmus* sp.*, Euglena* sp., *Chlamydomonas* sp., *Oscillatoria* sp., *Chlorella* sp., *Navicula* sp., *Botryococcus* sp., and *Stigeoclonium* sp. are eight genera in order of their tolerance to organic pollutants and adsorb nutrients such as nitrogen and phosphorus which also are able to treat wastewater. Some researchers

such as Kim, Chang & Acreman (2007), Khotari *et al.*, (2012), Abou-Shanab *et al.*, (2013), Hultberg *et al.*, (2013) and Jais *et al.*, (2015) succeeded in their research to remove nutrients by using microalgae while *Scenedesmus* sp. are known as ammonia tolerant algae (Lekshmi *et al.*, 2015; Miyazaki *et al.*, (1985).

In Malaysia, there are five location were used membrane biofilter in WTP as technology to treat WMWW in Kuala Lumpur, Malaysia which are Pasar Harian Selayang, Pasar Borong Kuala Lumpur, Pasar Jalan Klang Lama, Pasar Air Panas and Pasar Sentul. Others, the WMWW in Malaysia are flow directly to the river or the sea without any treatment. Besides that, in Philippines, fresh market wastewater in Muntinlupa City Market was treated by up-flow anaerobic sludge blanket (UASB) with additional of cocopeat filter. San Fernando Public Market in San Fernando City at La Union, Philippines was run a small-scale wastewater treatment system using UASB with additional chlorination tank to kill remaining pathogens or bacteria in the wastewater. Apart from that, Sta. Ana Public Market in Manila City, Metro Manila, Philippines also use UASB and chlorination chamber to treat the WMWW. However, the Sta. Ana Public Market which is alongside the Pasig River discharges the partial treated wastewater to the river, increase the pollution in Pasig River which known as most polluted river in the world. Above and over that, there are no technology was been used to treat WMWW for other public market. In worldwide, most of public markets are intentionally built near the river to make it easier to flow the wastewater directly to the river or sea without any treatment.

On the basis of this idea, microalgae *Scenedesmus* sp. was chosen as media to treat WMWW at Rengit Public Market. The batch experiment was held for optimization for removal of nutrients and organic contents from WMWW and microalgae *Scenedesmus* sp. bioreactor was designed as continuous experiment which like onsite situation.

1.2 Problem statement

Human activities in wet market such as poor segregation of fruits and vegetables, the sullage of fresh foodstuff, waste scraps of poultry and meat cuts after slaughtering, and fish entrails have led to significant increase in the massive production of undesirable wastes (Ghani, 2011; Yuliwati *et al.*, 2012; Zulkifli, Roshadah and Tunku Khalkausar,

2012; Al-Gheethi *et al.*, 2013; Efaq, Al-Gheethi & Mohamed, 2016; Mohamed *et al.*, 2016). These wastes will flow with water from the continuous washing of floor into the drainage. As a result, the disposed wastewater becomes rich with organic and inorganic residues (Abdel Raouf, Al-Homaidan & Ibraheem, 2012; Zulkifli *et al.*, 2012). The discharge of this untreated wet market wastewater indirectly causes serious problem to wastewater quality. If the wastewater eventually ends in the ponds, it can lead to eutrophication which adversely impact aquatic ecosystem, human health and visual disturbance (Amirkolaie, 2008; Zulkifli *et al.*, 2012). This issue has been debated due to the reduction of contaminants up to environmentally safe level (Martinez *et al*., 2000, Orpez *et al.*, 2009) with removal of nutrients as one of the major requirements in wastewater treatment process (Chowdhury, Viraraghavan, & Srinivasan, 2010; Abdel Raouf *et al.*, 2012).

Similar problem also occurs at Rengit Public Market in Batu Pahat, the biggest wet market and popular among the residents. This market has discharged a highly polluted wastewater directly into the nearest river without any treatment. Some activities in WMWW may cause stagnant wastewater, inviting flies because of the foul odour and thus creating uncomfortable view to buyers. Although green, the water at the coastal bank of the river is unhealthy because the green colour of the water is the results of eutrophication process. Typically, the WMWW is rich with nitrogen, phosphorus and carbon. The presence of these nutrients favours in the generation or growth of plants and algae (eutrophication). Eutrophication is known to give severe effect to water bodies, and then to environment. This chain of effect might eventually harm us too.

Therefore, more strategies are needed, and a sustainable method and approach is crucial in addressing this problem. Recently, phycoremediation process defined as utilization of microalgae for the treatment of wastewater pollution is promising. This technology exhibits high efficiency for wastewater quality improvement, especially in the presence of nitrogen and phosphorus that play important role in microalgae growth (Mcelwee, Baker & Clair, 2006; Prabakaran & Ravindran, 2012a). Hence in this research, phycoremediation technology using *Scenedesmus* sp. which is never been used in WMWW treatment was selected as solution for discharging problems occurred in Rengit Public Market, Batu Pahat. Rengit Public Market, Batu Pahat was chosen as sampling location because this public market is the second highest in term of food stuff loads, operation time, and the high numbers of buyers come to the public market.

Thus, the batch experiment was monitored and one system which is microalgae *Scenedesmus* sp. bioreactor were designed for continuous experiment to find the optimum result of initial inoculum of microalgae and minimum remaining of pollutant using the bioreactor. This phycoremediation technology offers an innovative step in exploring the potentials of microalgae *Scenedesmus* sp. as an alternative solution to be implemented in WMWW treatment in Malaysia.

1.3 Objectives of the research

The main aim of the research is to develop a prototype of *Scenedesmus* sp. bioreactor for phycoremediation process that capable in producing good quality of effluent and ensure the parameters in WMWW can reach allowable standard. This is followed by specific objectives to:

i. optimize the removal of nutrients and organic contents from WMWW by using different initial inoculum of microalgae *Scenedesmus* sp. in the agitated or unagitated condition in batch test approach.
ii. design a prototype of *Scenedesmus* sp. bioreactor for the removal of nutrients and organic contents from WMWW.
iii. investigate the performance of bioreactor in term of nutrients and organic contents from WMWW by *Scenedesmus* sp. bioreactor.
iv. evaluate the Michaelis-Menten biokinetic model for nutrient uptake by *Scenedesmus* sp. from WMWW.

1.4 Scope of the research

The research consists of field sampling, laboratory works and analysis with mathematical modelling during analysis. The field sampling of WMWW was located at Rengit Public Market, Batu Pahat, Johor. The microalgae *Scenedesmus* sp. obtained from tropical rainforest, Endau Rompin National Park, Johor was first being isolated in laboratory. The inoculation of microalgae colonies was provided and previously identified by the Microbiology Laboratory in the Faculty of Applied Science and Technology.

The characteristics of WMWW such as pH, temperature, total suspended solids (TSS) were investigated. These include evaluation of nutrients (Orthophosphate [PO_4^{3-}] and Ammonia Nitrogen [NH_3-N]) and organic contents (Biochemical Oxygen Demand [BOD] and Chemical Oxygen Demand [COD]) at laboratory using specific equipments such as Oakton pH 700 Benchtop Meter 'all in one' pH Electrode (pH 700 Oakton, USA), Ion Chromatography (Dionex), Buchi Speed Digester K-436 and Buchi Distillation K-355, BOD Incubator and DR 6000 Spectrometer (UV-VIS Hach, USA) and methods from Standard Method APHA 2012, and determination of peak hour operation time as optimum sampling time.

The optimization was performed in three steps of laboratory batch experiment: Primary treatment, secondary treatment, and tertiary treatment. Primary treatment of WMWW used equalization tank and screening process. Then, secondary treatment was followed by treating WMWW under two different conditions which are agitated and unagitated condition for three days to measure the highest reduction of organic contents. The best condition was chosen to be used for this stage. Phycoremediation process involving microalgae *Scenedesmus* sp. was continued in the next process as tertiary treatment. The optimum microalgae growth and nutrient removal were evaluated by adding four different inoculum cells of microalgae with two different conditions: agitated or unagitated within seven days in batch experiment.

Six days of phycoremediation during optimization was chosen to ensure that the development of *Scenedesmus* sp. bioreactor for removal of nutrients and organic contents from WMWW (as continuous experiment) were run smoothly. The microalgae *Scenedesmus* sp. bioreactor for removal of nutrients and organic contents from WMWW consisted of screen mesh steel, secondary treatment tank, two phycoremediation tanks and biomass container including auto sensors to detect time and amount of water level. Biomass container was used as medium to filter microalgae *Scenedesmus* sp. harvesting as dewatering technique which retain the microalgae on the filter become thick paste and allow the effluent to pass through the filter. The removal percentage of nutrients and organic contents from WMWW by *Scenedesmus* sp. bioreactor, and the uptake capacity rate of *Scenedesmus* sp. on ammonia-nitrogen (NH_3-N) and orthophosphate (PO_4^{3-}) by Michaelis-Menten biokinetic modelling were determined.

1.5 Hypothesis of the research

The following are some hypotheses of this research:

i. The WMWW is rich with nutrients and organic content such as nitrogen, phosphorus, BOD and COD.
ii. The agitated or unagitated condition have relationship on decreasing pollutant parameters such as BOD and COD as well as nutrients.
iii. The different initial inoculum of microalgae *Scenedesmus* sp. with agitated or unagitated condition in batch experiment have relationship on nutrient removal and specific growth rate which gives different results for each inoculum.
iv. The optimum results on nutrient removal and specific growth rate during batch experiment provide best inoculation of microalgae.
v. The performance of microalgae *Scenedesmus* sp. bioreactor for removal of nutrients and organic contents from WMWW prove the efficiency removal and microalgae growth during batch experiment.
vi. The effluent phycoremediation process is expected to comply the effluent discharge standard.

Therefore, the development of *Scenedesmus* sp. bioreactor prototype for removal of nutrients and organic contents from WMWW and the testing application of this system are important in providing a good system as wet market wastewater treatment alternative.

1.6 Significance of the research

Phycoremediation by microalgae *Scenedesmus* sp. in Rengit Public Market, Batu Pahat were chosen because of the wastewater type which contain high nutrients. The design and testing performance of *Scenedesmus* sp. bioreactor for the removal of nutrients and organic contents from WMWW is important in providing new insights on environmental friendly approach. Malaysia has no definite standard of WMWW. The Environmental Quality (Sewage) Regulations 2009 are used to refer more on effluent discharge into any inland water rather included (WMWW) than the effluent discharged

from prescribed premises. Hence, findings from this study are beneficial to authorities to produce a national guiding principle of WMWW treatment. Also, to wet market management and future researchers so that they can develop this strategy to overcome WMWW problem.

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